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The Influence of Bilirubin, Haemolysis and Turbidity on 20 Analytical Tests Performed on Automatic Analysers

Results of an interlaboratory study

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Summary: The director of a laboratory has to be sure to give out reliable results for routine tests on automatic analysers regardless of the clinical context. However, he may find hyperbilirubinaemia in some circumstances, parenteral nutrition causing turbidity in others, and haemolysis occurring if sampling is difficult. For this reason, the Commission for Instrumentation of the Société Française de Biologie Clinique (SFBC) (president *Alain Feuillu*) decided to look into “visible” interferences – bilirubin, haemolysis and turbidity – and their effect on 20 major tests: 13 substrates/chemistries: albumin, calcium, cholesterol, creatinine, glucose, iron, magnesium, phosphorus, total bilirubin, total proteins, triacylglycerols, uric acid, urea, and 7 enzymatic activities: alkaline phosphatase, alanine aminotransferase, α -amylase, aspartate aminotransferase, creatine kinase, γ -glutamyl transferase and lactate dehydrogenase measured on 15 automatic analysers representative of those found on the French market (Astra 8, AU 510, AU 5010, AU 5000, Chem 1, CX 7, Dax 72, Dimension, Ektachem, Hitachi 717, Hitachi 737, Hitachi 747, Monarch, Open 30, Paramax, Wako 30 R) and to see how much they affect the accuracy of results under routine conditions in the laboratory.

The study was carried out following the SFBC protocol for the validation of techniques using spiked plasma pools with bilirubin, ditauro-bilirubin, haemoglobin (from haemolysate) and IntralipidTM (turbidity).

Overall, the following results were obtained: haemolysis affects tests the most often (34.5% of cases); total bilirubin interferes in 21.7% of cases; direct bilirubin and turbidity seem to interfere less at around 17%. The different tests are not affected to the same extent; enzyme activity is hardly affected at all; on the other hand certain major tests are extremely sensitive, increasingly so as we go through the following: creatinine (interference of bilirubin), triacylglycerols (interference of bilirubin and haemoglobin), glucose (interference of bilirubin), cholesterol (interference of bilirubin), phosphorus (interference of bilirubin and haemoglobin), uric acid (interference of turbidity), iron (interference of haemoglobin and turbidity), total proteins (interference of bilirubin, haemoglobin and turbidity) and bilirubin (interference of haemoglobin and turbidity). Three categories of interferences can be found: interference by addition, chemical interference and spectral interference, and the results show that not only the choice of a method is important on the analyser, but also how this has been adapted.

By looking into these conditions carefully, it is sometimes possible to find a reason for the problem and thereby a simple solution to correct it. If the different factors are defined with care in terms of the reaction, use of a sample blank or not, choice of secondary wavelength etc., the influence of the interferences can be better kept under

control. During the study we have particularly noted how poorly effective bichromatic procedures are; the choice of the secondary wavelength did not always prove useful, and thus the "corrections" were often not effective or only slightly so, or even had the contrary effect.

1 Introduction

In 1991, the committee for instrumentation of the Société Française de Biologie Clinique (SFBC), president *Alain Feuillu*, decided to study the influence of "visible"

1) Enzymes

α -Amylase:

α -1,4-glucan 4-glucanhydrolase (EC 3.2.1.1)

Alanine amino transferase:

L-alanine : 2-oxoglutarate aminotransferase (EC 2.6.1.2)

Alkaline phosphatase:

orthophosphoric monoester phosphohydrolase (alkaline optimum) (EC 3.13.1)

Aspartate amino transferase:

L-aspartate : 2-oxoglutarate aminotransferase (EC 2.6.1.1)

Creatine kinase:

ATP : creatine N phosphotransferase (EC 2.7.3.2)

γ -Glutamyl transferase:

γ -glutamyl transpeptidase (EC 2.3.2.2)

Lactate dehydrogenase:

L-lactate : NAD⁺ oxidoreductase (EC 1.1.1.27)

interferences – bilirubin, haemolysis, turbidity – on the major routine tests carried out on automatic analysers. In many laboratories today, directors are often unaware of this type of problem. This led the committee to study how much these interferences affect the accuracy of results obtained for 20 routine analytes.

The results were presented at a workshop held in Euro-lab (Nice – France) on the 29 April 1993.

2 Materials and Methods

2.1 Instruments and analytes

The instruments included in the study consisted of a sample representative of those found on the French market. The list was compiled with the aid of manufacturers and distributors as well as in collaboration with colleagues in the profession. We tried to have 2 sites for each instrument when possible. Table 1 lists the instruments evaluated with the corresponding sites.

Tab. 1 Instruments tested and evaluation sites.

Manufacturer	Instrument	Evaluation site	Person in charge
Beckman	Astra	(1) CHLS – Secteur Ste Eugénie – Lyon (2) CHLS – Secteur J. Courmont – Lyon	R. C. Boisson M. Manchon
Merck	AU 510	(1) CH de Martigues (2) CH de Thonon	P. Desvignes F. Joncquiert
	AU 5010 AU 5000	(1) Centre de Méd. Prév. Nancy (2) CHLS – Secteur J. Courmont – Lyon	F. Schiele–J. Henny M. C. Carlier–P. Baltassat
Bayer	Chem 1	(1) HIA Desgenettes – Lyon (2) CH de Thionville	Y. Robet B. Capolaghi
Beckman	CX 7	(1) CHLS – Secteur J. Courmont – Lyon (2) CH de Meaux	M. Manchon Y. Gourmelin
Bayer	Dax 72	(1) GH Sud du CHR de Bordeaux	R. Crockett
Dupont	Dimension	(1) Hôp. F. WIDAL – Paris (2) CH de Nemours	C. Caliez–P. Levillain J. Morice–M. Porcheron
Kodak	Ektachem	(1) CHU de Rangueil – Toulouse (2) HIA Begin – Saint Mandé	M. Lagente–P. Valdiguié J. P. Yvert
Boehringer	Hitachi 717	(1) CHU Pontchaillou – Rennes (2) CH de Thionville (3) Soc. Boehringer – Meylan	A. Feuillu B. Capolaghi M. C. Lotz–C. Metier
	Hitachi 737 Hitachi 747	(1) HIA Begin – Saint Mandé (1) CH de Mulhouse	J. P. Yvert M. Dorr–C. Merzullo–M. O. Michotey
Instrumentation Laboratory	Monarch	(1) Hôp. Ste Marguerite – Marseille (2) CH de Limeil Brevannes	A. M. Pauli S. Troupel–C. Laureaux
Biomérieux	Open 30	(1) Hôp. Croix-Rousse – Lyon (2) CHLS – Secteur Ste Eugénie – Lyon	M. Bondon–D. Grafmeyer R. C. Boisson
Baxter	Paramax	(1) CH de Douai (2) GH sud du CHR de Bordeaux	P. Fievet–J. J. Kapoun R. Crockett
Serono	Wako 30 R	(1) HIA Desgenettes – Lyon	Y. Robet

The following analytes were studied: 13 substrates/chemistries, albumin, calcium, cholesterol, creatinine, glucose, iron, magnesium, phosphorus, total bilirubin, total proteins, triacylglycerols, uric acid and urea; and 7 enzymatic activities: alkaline phosphatase, alanine aminotransferase, α -amylase, aspartate aminotransferase, creatine kinase, γ -glutamyl transferase and lactate dehydrogenase.

Of course, for closed systems, Astra, Chem 1, one part of the CX 7, Dimension, Ektachem, Paramax, the methods used are those provided by the manufacturers; for open systems, the methods used are described in table 2 for chemistries and table 3 for enzyme activities measurements.

2.2 Materials

Bilirubin was obtained from Fluka (Mulhouse, France); conjugated bilirubin (sodium salt of ditau-bilirubin) from Porphyrin Products (Paris, France); IntralipidTM (a 20% fat emulsion for intravenous injection) from Kabi Pharmacia (Paris, France).

2.3 Plasma pools

Plasma pools were made up as carefully as possible to ensure the best results, i. e. trying to have concentrations of the analytes that

will easily show the apparent increases or decreases due to the various additions made (tab. 4). Also, we asked the evaluators to avoid using pools that already show haemolytic, icteric or lipaemic aspects. This implied preparing as many as 7 different pools.

2.4 Preparation of spiked solutions

Starting from freshly prepared stock solutions of bilirubin 5.1 mmol/l in 0.025 mmol/l NaOH, ditau-bilirubin 5.1 mmol/l in distilled water, plasma pools were prepared containing bilirubin concentrations between 0 and 500 μ mol/l, and ditau-bilirubin concentrations between 0 and 250 μ mol/l. These pools were analysed immediately. Using a solution of haemolysed erythrocytes in distilled water, plasma pools were prepared containing haemoglobin concentrations between 0 and 240 μ mol/l; the concentration of haemoglobin was tested for all samples. Intralipid was added to plasma pools in order to obtain turbidity, tested by measuring the absorbance at 600 nm of a 1 : 20 dilution in 150 mmol/l NaCl of the spiked plasma pools (turbidity index), equivalent to those of fresh sera with a concentration of triacylglycerols up to 7 mmol/l.

2.5 Protocol, procedure

The study was carried out using the SFBC protocol for the validation of techniques ("VALTEC") (1), in particular, appendix 9. The

Tab. 2 Methods used for chemistries determinations on open systems.

Analyte	Method group	Instrument	Reagent source
Albumin	Bromocresol green	CX 7 (2) Hitachi 717 (1) Monarch (1) Wako 30 R	Biomerieux Boehringer Instrumentation Lab. Serono
Calcium	<i>o</i> -Cresolphthalein complexone	AU 510 – AU 5010 (1) – AU 5000 (2) Dax 72 Hitachi 717 (1), (2), (3) – Hitachi 747 Monarch (1), (2) Wako 30 R	Merck Bayer Boehringer Instrumentation Lab. Serono
	Arsenazo III	Hitachi 737	Kone
Cholesterol	Cholesterol oxidase/peroxidase	AU 510 (1), (2) – AU 5010 (1) CX 7 (2) Dax 72 Hitachi 717 (2) – Hitachi 737 – Hitachi 747 Monarch (1), (2) Open 30 (1), (2) Wako 30 R	Merck Biomerieux Bayer Boehringer Instrumentation Lab. Biomerieux Serono
Creatinine	<i>Jaffé</i> kinetic	AU 510 (1) – AU 5000 (2) Dax 72 Hitachi 717 (2), (3) – Hitachi 737 – Hitachi 747 Monarch (1), (2) Wako 30 R	Merck Bayer Boehringer Instrumentation Lab. Serono
	Enzymatic	Hitachi 717 (1)	Boehringer
Glucose	Glucose oxidase/peroxidase	AU 510 (2) – AU 5000 (2) Dax 72 Hitachi 717 (1), (2) – Hitachi 737 – Hitachi 747 Monarch (1), (2) Wako 30 R	Merck Bayer Boehringer Instrumentation Lab. Serono
Iron	Ferene	Hitachi 717 (2) – Hitachi 737 Monarch (1)	Kone Instrumentation Lab.
	Ferrozine	AU 510 (1), (2) CX 7 (2) Hitachi 717 (3) Hitachi 747 Open 30 (2) Wako 30 R	Merck Biomerieux Boehringer Merck Biomerieux Serono

Tab. 2 (continued)

Analyte	Method group	Instrument	Reagent source
Magnesium	Calmagite	CX 7 (1) CX 7 (2) Hitachi 747	Beckman Biomerieux Biomerieux
	Magon	AU 510 (1), (2) – AU 5000 (2) Wako 30 R	Merck Serono
Phosphorus	Phosphomolybdate	AU 510 (1) – AU 5010 (1) – AU 5000 (2) AU 510 (2) CX 7 (1), (2) Dax 72 Hitachi 717 (1) Hitachi 717 (2) – Hitachi 737 – Hitachi 747 Monarch (1) Open 30 (1), (2) Wako 30 R	Merck Biomerieux Biomerieux Bayer Biomerieux Boehringer Instrumentation Lab. Biomerieux Serono
Proteins	Biuret with iodide	AU 510 (1) – AU 5010 (1) Hitachi 717 (1), (2) – Hitachi 737 – Hitachi 747 Monarch (1), (2) Wako 30 R	Merck Boehringer Instrumentation Lab. Serono
	Biuret without iodide	CX 7 (1), (2) Dax 72	Beckman Bayer
Total bilirubin	<i>Jendrassik</i> (benzoate caffeine) – neutral pH	AU 5000 (2) CX 7 (1), (2)	Merck Beckman
	– alkaline pH	Hitachi 717 (2), (3) – Hitachi 737	Boehringer
	<i>Jendrassik</i> (DMSO), neutral pH	Open 30 (1), (2)	Biomerieux
	Diphenyl diazonium (DPD)	AU 510 (1), (2) – AU 5010 (1) Hitachi 717 (1) – Hitachi 747	Merck Boehringer
	Others diazo reagents	Dax 72 Monarch (1), (2) Wako 30 R	Bayer Instrumentation Lab. Serono
Triacylglycerols	Enzymatic 340 nm	Open 30 (1)	Biotrol
	Enzymatic <i>Trinder</i>	AU 510 (1), (2) – AU 5010 (1) CX 7 (2) Dax 72 Hitachi 717 (2) – Hitachi 737 – Hitachi 747 Monarch (1), (2) Open 30 (2) Wako 30 R	Merck Biomerieux Bayer Boehringer Instrumentation Lab. Biomerieux Serono
Urea	Urease/glutamate dehydrogenase	AU 510 (1) – AU 5000 (2) Dax 72 Hitachi 717 (1) – Hitachi 737 – Hitachi 747 Hitachi 717 (2) Monarch (1) Wako 30 R	Merck Bayer Boehringer Biomerieux Instrumentation Lab. Serono
Uric acid	Uricase/catalase/dehydrogenase	AU 510 (2) AU 5000 (2) CX 7 (1) CX 7 (2) Open 30 (1), (2)	Biomerieux Merck Biotrol Biomerieux Biotrol
	Uricase/oxidase	AU 510 (1) – AU 5010 (1) Dax 72 Hitachi 717 (1), (2) – Hitachi 737 – Hitachi 747 Monarch (1), (2) Wako 30 R	Merck Bayer Boehringer Instrumentation Lab. Serono

Abbreviations

DMSO = Dimethyl sulphoxide

Tab. 3 Methods used for enzymatic activities measurement on open systems.

All systems work at 37 °C except CX 7 (2), Hitachi 717 (3), Hitachi 737, Monarch (1) and (2) which work at 30 °C.

Analyte	Method group	Instrument	Reagent source
Alanine amino-transferase and aspartate amino-transferase	Acc. to SFBC/IFCC (with pyridoxal-5-phosphate)	AU 510 (2)	Biomerieux
		AU 5000 (2)	Merck
		CX 7 (1), (2)	Biomerieux
		Hitachi 717 (2)	Biomerieux
		Open 30 (1), (2)	Biomerieux
	Others, with pyridoxal-5-phosphate	Dax 72	Bayer
Alkaline phosphatase	Acc. to SFBC/IFCC (without pyridoxal-5-phosphate)	AU 510 (1)	Merck
		Hitachi 717 (1) – Hitachi 737 – Hitachi 747	Boehringer
		Monarch (1), (2)	Instrumentation Lab.
		Wako 30 R	Serono
		AU 510 (2)	Biomerieux
		AU 5010 (1)	Boehringer
	Acc. to DGKC (with diethanolamine buffer)	AU 5000 (2)	Merck
		CX 7 (1), (2)	Biomerieux
		Dax 72	Bayer
		Hitachi 717 (2) – Hitachi 737 – Hitachi 747	Boehringer
		Open 30 (1)	Boehringer
		Open 30 (2)	Biomerieux
α -Amylase	Maltotetraose PNP-G7	AU 510 (1)	Merck
		AU 510 (2)	Biomerieux
		CX 7 (1)	Biomerieux
		Hitachi 717 (1) – Hitachi 747	Boehringer
		Hitachi 717 (2) – Hitachi 737	Biomerieux
		Monarch (1)	Instrumentation Lab.
		Open 30 (1), (2)	Biomerieux
		Wako 30 R	Serono
		AU 510 (1) – AU 5000 (2)	Merck
		AU 510 (2)	Biomerieux
Creatine kinase	N-Acetylcysteine activated, acc. to DGKC/SFBC	CX 7 (1), (2)	Biomerieux
		Dax 72	Bayer
		Hitachi 717 (1)	Biomerieux
		Hitachi 717 (2) – Hitachi 737 – Hitachi 747	Boehringer
		Monarch (1)	Instrumentation Lab.
		Open 30 (1), (2)	Biomerieux
	N-Acetylcysteine activated, others	Wako 30 R	Serono
		Hitachi 717 (2)	Eurodiag.
		Monarch (1)	Instrumentation Lab.
		AU 510 (2), (2) – AU 5010 (1) – AU 5000 (2)	Merck
γ -Glutamyl transferase	Carboxylated substrate, acc. to IFCC	Hitachi 717 (1), (3) – Hitachi 737 – Hitachi 747	Boehringer
	Carboxylated substrate, others	CX 7 (1), (2)	Biomerieux
	Non-carboxylated substrate	Dax 72	Bayer
		Open 30 (1), (2)	Biomerieux
		Wako 30 R	Serono
Lactate dehydrogenase	Acc. to DGKC	AU 510 (1)	Merck
		Hitachi 717 (1) – Hitachi 747	Boehringer
		Monarch (1)	Instrumentation Lab.
		AU 510 (2) – AU 5000 (2)	Biomerieux
	Acc. to SFBC	CX 7 (1)	Biotrol
		Hitachi 717 (2)	Biomerieux
		Hitachi 737	Boehringer
		Open 30 (1)	Biotrol
		Open 30 (2)	Biomerieux
	Others	CX 7 (2)	Beckman

AbbreviationsSFBC = Société Française de Biologie Clinique
IFCC = International Federation of Clinical ChemistryDGKC = Deutsche Gesellschaft für Klinische Chemie
PNP-G7 = *p*-nitrophenyl maltoheptaoside

Tab. 4 Range of concentration or activity of plasma pools to be spiked.

For enzyme activities, expressed in multiples of the upper limit of reference values (N).

	Unit	Range of concentration or activity
Albumin	g/l	25 to 40
Calcium	mmol/l	2.00 to 2.50
Cholesterol	mmol/l	4.0 to 5.0
Creatinine	μmol/l	200 to 250
Glucose	mmol/l	5.0 to 7.0
Iron	μmol/l	15.0 to 25.0
Magnesium	mmol/l	0.75 to 1.00
Phosphorus	mmol/l	1.00 to 1.50
Proteins	g/l	55 to 75
Total bilirubin	μmol/l	40 to 50
Triacylglycerols	mmol/l	1.20 to 1.80
Urea	mmol/l	12.0 to 18.0
Uric acid	μmol/l	200 to 300
α-Amylase	N	1.5 to 2.5
Alanine amino transferase	N	1.5 to 2.5
Alkaline phosphatase	N	1.5 to 2.5
Aspartate amino transferase	N	1.5 to 2.5
Creatine kinase	N	1.5 to 2.5
γ-Glutamyl transferase	N	1.5 to 2.5
Lactate dehydrogenase	N	1.5 to 2.5

evaluators were asked to pay special attention to the following points: running the tests in duplicate and adding the supplement material just prior to testing to avoid any transformation of the interfering substance.

2.6 Calculation of results

Final results were calculated by MacVALTEC 3.0 software (J. C. Eynard, D. Grafmeyer) running on an Apple Macintosh worksta-

tion on the basis of the measurements' average. If the results from 2 sites did not agree, i.e. if the difference between the two results was greater than or equal to twice the used acceptable limit (tab. 5), they were rechecked (of 83 values involved, 40 were corrected) by the two involved sites.

3 Results

3.1 Results by instrument

Firstly, we drew up "interferographs" as indicated in the VALTEC protocol with the concentration of interference on the x-axis (concentrations of bilirubin and haemoglobin, turbidity index), and the difference between the results of the pool and the spiked specimen expressed in % of the pool concentration on the y-axis.

As stated above, we split the tests into 2 groups, "chemistries" and "enzymes". Taking the 4 different interferences into consideration (bilirubin and ditauro-bilirubin, haemolysis and turbidity), we find we have 1 or 2 graphs per interference per group, i.e. 12 graphs per analyser which leads to more than 250 graphs in total. On each graph, we have indicated the "acceptable limits" as given by the VALTEC protocol (tab. 3). Note, however, that the protocol does not stipulate values for albumin or amylase.

Having drawn up the graphs, we then summarised the data in the form of 3 tables (tab. 6, 7, 8). Table 6 shows the results for total and ditauro-bilirubin; note the relationship between the 2 types of interference, the lack of interference for enzyme activities measurements, and above the very frequent interference shown for cholest-

Tab. 5 Acceptable limits (according to SFBC-VALTEC guidelines) except for albumin and α-amylase.

	Unit	Concentration or activity	VALTEC norms	
			in units	as %
Albumin	g/l	40	4	10.0
Calcium	mmol/l	2.40	0.10	4.2
Cholesterol	mmol/l	5.00	0.50	10.0
Creatinine	μmol/l	140	15	10.7
Glucose	mmol/l	6.0	0.5	8.3
Iron	μmol/l	20.0	1.5	7.5
Magnesium	mmol/l	0.80	0.08	10.0
Phosphorus	mmol/l	1.30	0.14	10.8
Proteins	g/l	65	3	4.6
Total bilirubin	μmol/l	30	5	16.7
Triacylglycerols	mmol/l	1.40	0.16	11.4
Urea	mmol/l	10.0	1.0	10.0
Uric acid	μmol/l	300	30	10.0
α-Amylase	U/l	100	15	15.0
Alanine amino transferase	U/l	100	5	5.0
Alkaline phosphatase	U/l	160	15	9.4
Aspartate amino transferase	U/l	50	5	10.0
Creatine kinase	U/l	100	15	15.0
γ-Glutamyl transferase	U/l	50	8	16.0
Lactate dehydrogenase	U/l	300	30	10.0

terol, creatinine, glucose, phosphorus, total proteins, triacylglycerols and uric acid determinations. Table 7 shows the results for haemolysis; note that haemolysis does interfere very frequently on chemistries determinations, particularly for iron, phosphorus, total proteins, bilirubin and uric acid as for the measurement of the activities of alkaline phosphatases and creatine kinase. Table 8 shows the results for turbidity. Note its less frequent interference; hardly ever on enzyme activities measurements, and only on some chemistries: e. g. glucose, iron, phosphorus, total proteins, bilirubin and uric acid.

3.2 Overall results per analyte

We also drew up "interferographs" per analyte with the results of several instruments for the same analyte, with the concentration of interference (concentrations of bilirubin and haemoglobin, turbidity index) of the x-axis, and the difference between the results of the pool and the spiked specimen expressed in concentration on the y-axis. Some representative examples of these graphs are shown (fig. 1, 2, 3).

In table 9, the results of the tests have been grouped together by analyte. The number of tests carried out (N) and the number of times an interference was seen at the highest level of addition are shown. This provides at least 2 types of information: the substance which interferes most often, and the analyte(s) which is (are) most sensitive to interference. In this way, 1443 tests were performed for which 324 interferences were noted, i. e. 22.5%. Haemolysis interferes the most often: in 348 tests, 120 were affected (34.5%). Total bilirubin interferes in 21.7% of cases (84 times out of 387 tests).

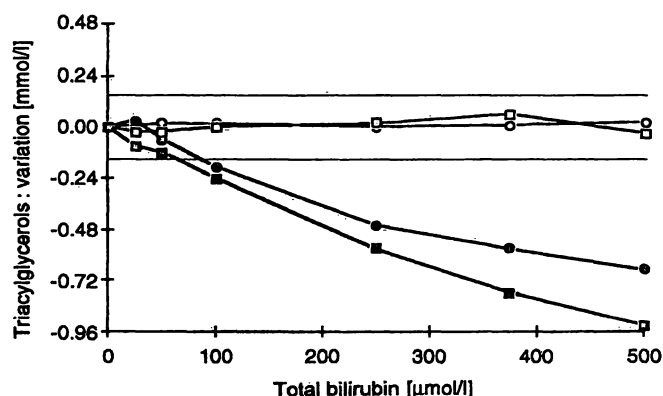


Fig. 1 Example of interferograph: the influence of bilirubin on triacylglycerols measurement.

In that case, the interference depends on the method used: with the enzymatic method with 340 nm readings on the Open 30 (1) and Chem (1) analysers, there is no interference; and with the enzymatic peroxidase methods on the Open 30 (2) et CX 7 (2) analysers, the interference is significant.

—○— Open 30 (1); —●— Open 30 (2); —□— Chem 1 (1); —■— CX 7 (2)

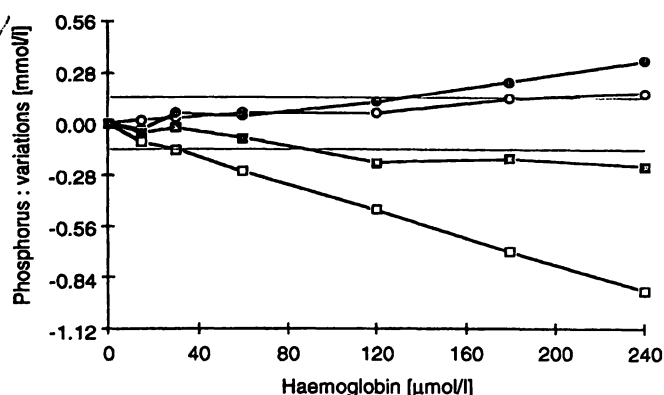


Fig. 2 Example of interferograph: the influence of haemolysis on phosphorus measurement.

All the analysers use the same method: phosphomolybdate with 340 nm readings; a close examination of the operating conditions may help to explain, at least partly, the results.

—○— AU 510 (1); —●— CX 7 (1); —□— Dimension (1); —■— Paramax (1)

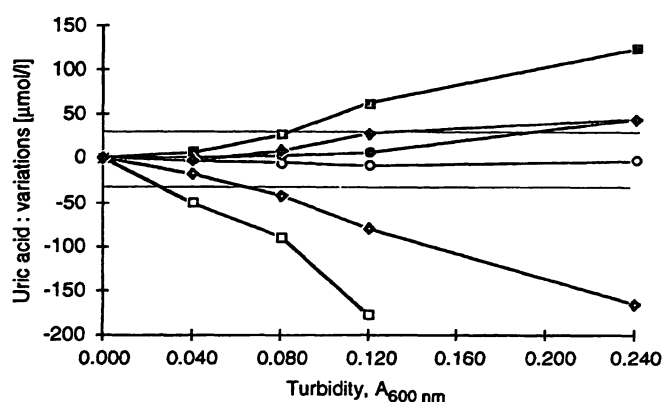


Fig. 3 Example of interferograph: the influence of turbidity on uric acid measurement.

The shown interference are not dependent on the methods: the Chem 1 (2) and the AU 510 (2) work with an "uricase/catalase/dehydrogenase" method, the other analysers with "uricase/peroxidase" methods.

—○— AU 510 (1); —□— Chem 1 (1); —◇— Monarch (2)
—●— AU 510 (2); —■— Dax 72; —◆— Paramax (1)

Ditauro-bilirubin causes less interference, 16.7% (53 of 317 tests), but the concentrations were not as high, 250 $\mu\text{mol/l}$ as opposed to 500 $\mu\text{mol/l}$ for total bilirubin. For turbidity, interference was seen in 67 of 391 tests (17.1%).

The sensitivity to interference was not the same for all analytes; they can be divided up into 3 groups:

- slightly sensitive or not at all sensitive: in this group, less than 10% of specimens showed interference according to the norms of the protocol: urea, α -amylase, γ -glutamyl transferase, lactate dehydrogenase and alkaline phosphatase.
- fairly sensitive: in this group, a significant interference was seen in 10 to 20% of cases noted as indicated in the protocol: albumin, calcium, magnesium, aspartate aminotransferase and creatine kinase.

Tab. 6 The influence of total (raw 1) and ditauuro-bilirubin (raw 2); highest concentration of addition, total bilirubin 500 µmol/l; ditauuro-bilirubin 250 µmol/l.

	Albu- min	Cal- cium	Chole- sterol	Crea- tinine	Glucose	Iron	Magne- sium	Phos- phorus	Pro- teins	Tri- acyl- glyce- rols	Uric acid	Urea	Ala- nine amino- trans- ferase	Aspar- tate amino- trans- ferase	Alka- line phos- phatase	α-Amy- lase	Crea- tine kinase	γ-gluta- myl- trans- ferase	Lac- tate de- hydro- genase
Astra 8	(1)	/	0	/	0	/	/	/	-6%	/	/	0	/	/	/	/	/	/	/
	(2)	/	0	/	0	/	/	/	0	/	/	0	/	/	/	/	/	/	/
AU 510	(1)	/	0	-31%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	(2)	/	0	-20%	0	0	0	+15%	0	-24%	-43%	0	0	0	0	0	0	0	0
AU 5010	(1)	/	0	-33%	/	0	0	+14%	/	+19%	0	/	0	0	0	0	0	0	0
	(2)	/	0	-19%	/	0	0	+41%	/	-45%	0	/	0	0	0	0	0	0	0
AU 5000	(1)	/	0	-42%	/	0	0	0	-16%	0	-11%	/	/	/	0	/	/	0	/
	(2)	/	0	-15%	/	0	0	0	-8%	-25%	-19%	/	/	/	0	/	/	0	/
Chem 1	(1)	0	0	-12%	0	0	0	0	0	/	-22%	0	0	0	0	/	0	0	0
	(2)	0	0	-25%	0	0	0	0	0	/	0	0	0	0	0	/	0	0	0
CX 7	(1)	0	0	-12%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	(2)	0	0	-12%	0	0	0	+12%	0	0	0	0	0	0	0	0	0	0	0
Dax 72	(1)	0	0	-25%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	(2)	0	0	-20%	0	0	0	+17%	0	-78%	0	0	0	0	0	0	0	0	0
Dimension	(1)	0	0	-16%	0	0	0	0	-6%	/	/	/	/	/	/	/	/	/	/
	(2)	0	0	-16%	0	0	0	0	-10%	/	/	/	/	/	/	/	/	/	/
Ektachem	(1)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	(2)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hitachi 717	(1)	-13%	-10%	0	-18%	/	/	-57%	-18%	/	-41%	0	0	0	0	0	0	0	0
	(2)	0	0	-35%	0	/	/	0	-8%	/	-23%	0	0	0	0	0	0	0	0
	(3)	0	0	-20%	-27%	+14%	/	0	-7%	0	-29%	0	0	0	0	0	0	0	0

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0 no significant interference seen at the highest level
Values in *italics* = significant interference (according to the norms) at the highest level of interference and the extent of this interference

Tab. 7 The influence of haemolysis; highest concentration of addition of haemoglobin 240 µmol/l.

	Albu- min	Cal- cium	Chole- sterol	Crea- tinine	Glu- cose	Iron	Magne- sium	Phos- phorus	Pro- teins	Total bili- rubin	Tri- acyl- glyce- rols	Uric acid	Urea	Alka- line phos- pha- tase	α-Amy- lase	Crea- tine kinase	γ-Glu- taryl- trans- ferase
Astra 8	(1) /	0	/	-12%	0	/	/	/	0	/	/	/	0	/	/	/	/
	(2) /	0	/	0	0	/	/	/	0	/	/	/	0	/	/	/	/
AU 510	(1) /	0	0	0	0	+81%	+12%	+16%	+12%	-43%	0	+47%	0	-18%	0	0	-22%
	(2) /	/	0	/	0	+64%	+16%	+58%	/	+55%	+17%	0	/	0	-28%	+20%	0
AU 5010	(1) /	0	-20%	/	0	/	/	-17%	0	0	+14%	+42%	/	-15%	/	/	0
AU 5000	(2) /	0	/	0	0	/	0	+21%	/	-60%	/	0	0	-45%	/	0	0
Chem 1	(1) 0	-7%	-13%	0	0	0	0	0	0	0	0	+13%	0	-11%	0	+40%	0
	(2) /	-7%	-15%	0	0	0	/	0	+6%	0	0	0	0	0	/	/	0
CX 7	(1) /	0	/	0	0	/	0	+29%	0	+47%	/	-11%	0	+16%	0	0	0
	(2) 0	0	0	0	0	-50%	+14%	+40%	0	+33%	0	0	0	0	0	+20%	??
Dax 72	(1) /	0	0	0	-19%	/	/	+17%	+11%	-36%	+14%	-31%	0	0	/	0	0
Dimension	(1) /	+5%	0	0	0	+187%	/	-77%	0	0	/	0	0	0	/	0	?
	(2) /	0	0	0	0	+150%	0	-78%	+7%	0	+57%	0	0	0	0	0	0
Ektachem	(1) /	0	0	0	-9%	/	/	+11%	+20%	+37%	0	0	0	0	0	0	0
	(2) /	0	0	0	0	-15%	0	0	+11%	+34%	0	0	0	+14%	0	0	0
Hitachi 717	(1) -12%	0	/	0	0	/	/	??	+8%	0	/	+15%	0	-18%	0	+41%	0
	(2) /	0	0	0	0	+140%	/	0	+6%	-49%	0	0	0	0	0	+23%	0
	(3) /	0	/	0	/	+94%	/	/	/	-50%	/	/	/	/	/	/	0
Hitachi 737	(1) /	0	0	0	0	+17%	/	0	+7%	-19%	0	+36%	0	0	0	0	0
Hitachi 747	(1) /	0	0	0	0	+13%	0	0	+7%	-78%	0	+18%	0	0	0	+28%	-27%
Monarch	(1) 0	+5%	0	0	0	+131%	/	+24%	+16%	0	0	+58%	0	-24%	0	0	0
	(2) /	0	0	0	0	/	/	/	+17%	0	0	+27%	/	/	/	/	/
Open 30	(1) /	/	0	/	/	/	/	+37%	/	-19%	0	0	/	0	0	+37%	0
	(2) /	/	0	/	/	0	/	+37%	/	-30%	0	-11%	/	0	0	0	0
Paramax	(1) 0	0	0	0	0	+62%	0	-17%	+7%	+75%	+21%	+16%	0	0	0	0	0
	(2) 0	0	0	-13%	0	+49%	0	-20%	+8%	+20%	+25%	+29%	0	??	/	+16%	??
Wako 30 R	(1) +20%	-21%	+51%	0	0	+295%	0	0	+39%	+543%	0	+215%	0	0	0	+265%	-27%

Values in italics = significant interference (according to the norms) at the highest level of interference

Tab. 8 The influence of turbidity; highest concentration of addition, equivalent to a concentration of triglycerides of 7 mmol/l.

	Albu- min	Cal- cium	Chole- sterol	Crea- tinine	Glucose	Iron	Magne- sium	Phos- phorus	Pro- teins	Total bili- rubin	Uric acid	Urea	Ala- nine amino- trans- ferase	Aspar- tate amino- trans- ferase	Alka- line phos- pha- tase	α - Amy- lase	Crea- tine kinase	γ - Gluta- myl trans- ferase	Lac- tate de- hydro- genase
Astra 8	(1) /	0	/	-12%	0	/	/	/	0	/	/	0	/	/	/	/	/	/	/
	(2) /	0	/	0	0	/	/	/	0	/	/	0	/	/	/	/	/	/	/
AU 510	(1) /	0	0	0	+20%	0	+17%	0	+17%	0	0	0	-14%	-15%	0	0	0	0	0
AU 510	(2) /	/	0	/	+30%	-100%?	+22%	+127%	/	0	+22%	/	0	0	0	0	-16%	0	0
AU 5010	(1) /	0	0	/	+12%	/	/	0	0	0	0	/	/	/	0	/	/	0	/
AU 5000	(2) /	0	/	0	0	/	0	0	/	0	+12%	0	0	0	0	/	0	0	0
Chem 1	(1) 0	0	0	-16%	0	0	0	0	0	0	-61%?	0	0	0	0	0	0	0	0
	(2) /	0	0	+16%	0	+15%	/	0	-7%	0	-50%?	0	0	0	0	/	/	0	/
CX 7	(1) /	0	/	0	0	/	0	+48%	0	+31%	-15%?	0	0	0	0	0	0	0	0
	(2) 0	0	/	0	0	-14%	0	+74%	0	+19%	-10%	0	0	0	0	0	0	0	0
Dax 72	(1) /	0	0	0	+24%	/	/	0	+22%	+25%	+50%	0	0	0	0	/	0	0	/
Dimension	(1) /	0	0	0	0	0	/	0	0	0	/	0	0	0	0	/	0	0	0
	(2) /	-5%	0	0	+10%	0	-17%	0	0	0	0	0	0	0	0	0	0	0	0
Ektachem	(1) /	0	0	0	0	/	/	0	0	0	0	0	0	0	0	0	0	0	0
	(2) /	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hitachi 717	(1) 0	0	0	0	+14%	/	/	+53%	0	-30%	0	0	0	0	0	0	0	0	0
	(2) /	0	0	0	0	+74%	/	0	0	0	0	0	0	0	0	0	0	0	0
	(3) /	0	/	0	/	0	/	/	/	0	/	/	/	/	/	/	/	/	/
Hitachi 737	(1) /	+5%	0	0	0	+13%	/	0	0	0	0	0	0	0	0	0	0	0	0
Hitachi 747	(1) /	0	0	0	0	>-100%	0	0	0	0	0	0	0	0	0	0	0	0	0
Monarch	(1) 0	+6%	+16%	0	0	+241%	/	+61%	+14%	+75%	0	0	0	0	0	0	0	0	0
	(2) /	0	0	0	+10%	/	/	/	+18%	+78%	-50%	/	/	0	/	/	/	/	/
Open 30	(1) /	/	0	/	/	/	/	+112%	/	0	0	/	0	0	0	0	0	0	0
	(2) /	/	0	/	/	+17%	/	+78%	/	0	0	/	0	-19%	0	0	0	0	0
Paramax	(1) 0	0	0	0	0	+9%	0	0	+6%	+35%	+19%	0	0	0	0	0	0	0	0
	(2) 0	0	0	0	0	0	0	0	+9%	+46%	+20%	0	0	+13%	0	/	0	0	0
Wako 30 R	(1) 0	0	0	0	+12%	+13%	0	0	+17%	+245%	0	0	0	0	0	0	0	0	/

Values in italics = significant interference (according to the norms) at the highest level of interference

Tab. 9 Quantitative analysis of results : N : number of tests carried out – n: number of interferences found (at the highest level of addition).

	Bilirubin		Ditaurobilirubin		Haemolysis		Turbidity		Total interferences	
	N	n	N	n	N	n	N	n	number	%
"Chemistry"										
Albumin	7	2	5	0	7	2	7	0	4	15.4
Calcium	24	2	20	0	24	5	24	3	10	10.9
Cholesterol	21	15	17	11	21	4	21	1	31	38.8
Creatinine	23	10	19	7	23	2	23	3	22	25.0
Glucose	24	13	20	6	24	2	24	8	29	31.5
Iron	17	4	13	1	17	14	17	10	29	45.3
Magnesium	12	1	9	1	12	3	12	3	8	17.8
Phosphorus	23	6	19	8	23	15	23	7	36	40.9
Proteins	22	12	18	4	22	15	22	8	39	46.4
Total bilirubin	/	/	/	/	25	17	25	9	26	52.0
Triacylglycerols	20	5	17	4	20	6	/	/	15	26.3
Urea	21	0	17	0	21	0	21	0	0	0.0
Uric acid	24	9	20	8	24	14	23	10	41	45.1
Enzyme										
α-Amylase	17	0	14	0	17	1	17	0	1	1.5
Alanine amino transferase	23	1	19	1	/	/	23	1	3	4.6
Alkaline phosphatase	23	0	19	0	23	8	23	0	8	9.1
Aspartate amino transferase	22	3	18	2	/	/	22	3	8	12.9
Creatine kinase	21	0	17	0	21	9	21	1	10	12.5
γ-Glutamyl transferase	24	0	20	0	24	3	24	0	3	3.3
Lactate dehydrogenase	19	1	16	0	/	/	19	0	1	1.9
Total	387	84	317	53	348	120	391	67	1443	
		21.7%		16.7%		34.5%		17.1%	324	22.5%

– very sensitive: in this group, interference occurred in more than 20% of cases: creatinine: 25% – triacylglycerols: 26.3% – glucose: 31.5% – cholesterol: 38.8% – phosphorus: 40.9% – uric acid: 45.1% – iron: 45.3% – total proteins: 46.4% – total bilirubin: 52.0%.

4 Discussion

4.1 What can be ascertained?

The interference can be placed into 3 categories: interference by addition, chemical interference and spectral interference.

4.1.1 Interferences by addition

In this case, visible interference occurs due to the addition of external substances which increase the apparent amount of analyte. Typically, haemoglobin falls into this category, being an indicator of red cell lysis (2, 3); the contents of the red cells will subsequently be found in the plasma, modifying its composition. Several of the analytes tested are affected in this way: red cells are rich in enzymes which will prevent correct readings if specimens are haemolysed (lactate dehydrogenase, transaminases). They are also rich in organic phosphate

esters; thus the added amount can increase by up to 20%; in practice, this is not always found.

Haemoglobin is potentially a rich source of iron. However, the iron-porphyrin bonding is stronger than the bonding for the usual transport protein (transferrin), which means that releasing iron for tests can be adapted to avoid interference. This appears to be the case, as although 82% of tests were affected, this had nothing to do with an increase in iron (fig. 4). Some tests were

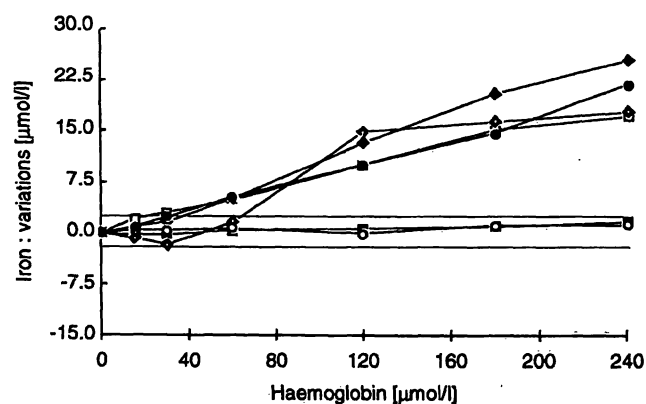


Fig. 4 The influence of haemolysis on iron measurement; because of its magnitude, the interference is not due to the release of iron from haemoglobin, but it probably has a spectral origin. –□– AU 510 (1); –◇– Hitachi 717 (3); –○– Open 30 (2) –■– Chem 1 (2); –◆– Dimension (1); –●– Hitachi 717 (2)

negatively influenced (2 cases, 2%). This suggests spectral interference.

Haemoglobin is also a protein which will also increase the plasma content in 68% of cases. Despite the fact that the added amount is small (maximum addition, 240 $\mu\text{mol/l}$, approximately 3.8 g/l for 70 g/l) this is still significant (protocol norm: 3 g/l for 65 g/l, tab. 5). In certain cases, the interference was increased even more due to additional spectral interference.

The concentration of erythrocytic magnesium is around 3 times greater than plasmatic values. This, therefore, causes increases in 25% of the cases, which is nevertheless negligible as the concentration of haemolysis needs to be far greater than the concentration used in the study to have an effect.

Creatinine kinase is strongly affected: in 43% of the cases the value increased, whereas with bilirubin and turbidity, virtually no difference can be seen. This is probably due to erythrocytic adenylate kinase which is not wholly inhibited under operating conditions (reagents contain adenosine monophosphate or diadenosine phosphate or a combination of the two).

4.1.2 Spectral interference

This is based on the law of added absorbances. All substances, other than the measured one, absorbing at the wavelengths used for the test will cause an apparent increase in the measured concentration. However, the substances included in the study have high absorbances

at many different wavelengths (fig. 5) and their spectra may change with the conditions (fig. 6 and 7).

Turbidity will interfere more at shorter wavelengths (Rayleigh's law, fig. 5). An example of this is uric acid measurement (tab. 10) using the uricase-catalase methods which suffer far more interference than the uricase/peroxidase methods (readings made at 340 nm and 505 nm, respectively). A further example is phosphorus measurement; nearly all methods use phosphomolybdate at 340 nm. This makes it sensitive to turbidity unless certain solutions are added (tab. 11). Catalytic activity of

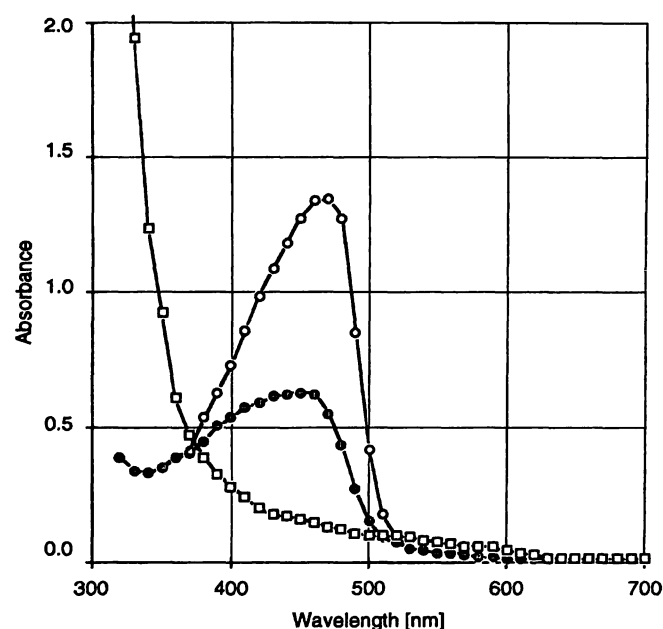


Fig. 6 The comparison of the absorbances of unconjugated bilirubin, conjugated, and unconjugated bilirubin in the presence of an oxidant H_2O_2 .

Same conditions as fig. 5.

—○— unconjugated bilirubin

—●— conjugated bilirubin

—□— unconjugated bilirubin in the presence of H_2O_2

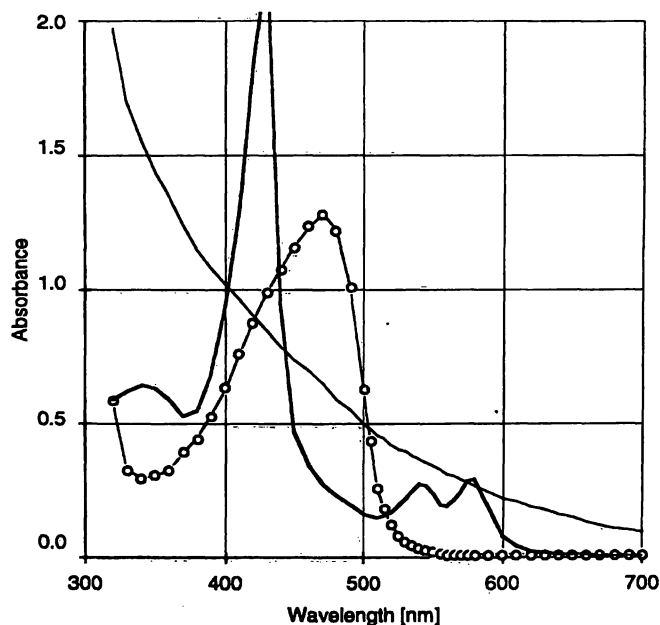


Fig. 5 The spectra of bilirubin, haemoglobin and turbidity compared; they were obtained on a Kontron Uvikon (model 810) double beam spectrophotometer with spiked pools at the highest level of addition, and then diluted 1 : 100 in 150 mmol/l NaCl.
—○— Bilirubin; —●— Haemoglobin; — Turbidity

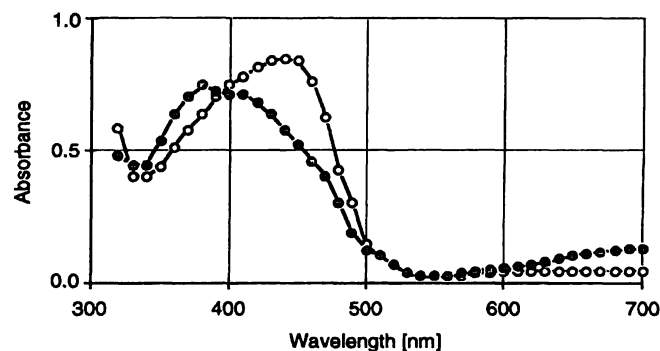


Fig. 7 Comparative absorbances of conjugated bilirubin at pH 1 and pH 7; at acid pH, there is a shift of the spectrum towards the shorter wavelengths.

NB: unconjugated bilirubin does not show differences at different pH values.

Same conditions as fig. 5.

—○— conjugated bilirubin at pH 7

—●— conjugated bilirubin at pH 1

Tab. 10 The influence of turbidity on uric acid measurement.

Method group	Instrument	Turbidity
Uricase/catalase/ dehydrogenase	AU 5000 (2)	+12%
	AU 510 (2)	+22%
	Chem 1 (1)	-61%?
	Chem 1 (2)	-50%?
	CX 7 (1)	-15%?
	CX 7 (2)	-10%
	Open 30 (1)	0
	Open 30 (2)	0
Uricase/oxidase	AU 5010 (1)	0
	AU 510 (1)	0
	Dax 72	+50%
	Hitachi 717 (1)	0
	Hitachi 717 (2)	0
	Hitachi 737	0
	Hitachi 747	0
	Monarch (1)	0
	Monarch (2)	-50%
	Paramax (1)	+19%
	Paramax (2)	+20%
	Wako 30 R	0
Uricase/reflectometry	Ektachem (1)	0
	Ektachem (2)	0
Uricase 293 nm	Dimension (1)	/
	Dimension (2)	0

Italic numbers = significant interference (according to the norms) at the highest level of interference

Tab. 11 The influence of turbidity on phosphorus measurement.

Method group	Instrument	Turbidity
Phosphomolybdate measurement at 340 nm	AU 5000 (2)	0
	AU 5010 (1)	0
	AU 510 (1)	0
	AU 510 (2)	+127%
	Chem 1 (1)	0
	Chem 1 (2)	0
	CX 7 (1)	+48%
	CX 7 (2)	+74%
	Dax 72	0
	Dimension (1)	0
	Dimension (2)	0
	Hitachi 717 (1)	+53%
	Hitachi 717 (2)	0
	Hitachi 737	0
	Hitachi 747	0
	Monarch (1)	+61%
	Open 30 (1)	+112%
	Open 30 (2)	+78%
	Paramax (1)	0
	Paramax (2)	0
	Wako 30 R	0
Reflectometry	Ektachem (1)	0
	Ektachem (2)	0

Italic numbers = significant interference (according to the norms) at the highest level of interference

enzyme measurement, on the other hand, is not affected unless the signal cannot be correctly measured when absorbance becomes too high at 340 nm.

Tab. 12 The influence of bilirubin on creatinine measurement for methods using the Jaffé reaction.

Instrument	Bilirubin	Ditauro-bilirubin	Measuring wavelengths (nm)
Astra 8 (1)	0	0	520
Astra 8 (2)	0	0	520
AU 5000 (2)	0	-12%	520/600
AU 510 (1)	0	0	520/570
Chem 1 (1)	-25%	-12%	500/600
Chem 1 (2)	-15%	-25%	500/600
CX 7 (1)	0	0	520/560
CX 7 (2)	-20%	/	520/560
Dax 72	-25%	-20%	500/604
Dimension (1)	-16%	/	510/700
Dimension (2)	-16%	0	510/700
Hitachi 717 (2)	0	0	505
Hitachi 717 (3)	-15%	-11%	505/570
Hitachi 737	0	-14%	505/570
Hitachi 747	0	/	505
Monarch (1)	0	0	520
Monarch (2)	0	0	520
Paramax (1)	+46%	0	525/630
Paramax (2)	0	0	525/630
Wako 30 R	+250%?	/	500/572

Italic numbers = significant interference (according to the norms) at the highest level of interference

Haemoglobin absorbs strongly at 415 nm ("Soret peak"), and less so between 530 and 600 nm, having 2 peaks at 540 and 570 nm (fig. 5) (2, 3). Therefore it may greatly interfere with measurements at these wavelengths (di-azo-bilirubin, biuret total proteins). However, the structure of haemoglobin varies with the medium, precipitating in acid media, converting to haematin in alkaline media, becoming reduced, forming methaemoglobin or even carboxy or sulphohaemoglobin. These all have different spectra which will modify the extent of interference.

Bilirubin has a high absorbance between 400 and 520 nm (see fig. 5) and may interfere with the measurement of creatinine using the Jaffé method (tab. 12). Apparently, using a secondary wavelength does not correct this effect.

4.1.3 Chemical interference

The presence of foreign molecules in the solution may cause interference at different stages of the reaction, consuming some of the products.

The effect of haemoglobin on bilirubin measurement has been well-defined and is typical of this type of interference (tab. 13, in 68% of cases). In this case the spectral interference must also be taken into account as azobilirubin is measured at 540 nm corresponding to one of the wavelengths at which oxyhaemoglobin absorbs. However, if the pH changes during the reaction, the

Tab. 13 The influence of haemoglobin on bilirubin measurement.

Method group	Instrument	Haemolysis
<i>Jendrassik</i> (benzoate caffeine) neutral pH	AU 5000 (2)	-60%
	CX 7 (1)	+47%
	CX 7 (2)	+33%
	Dimension (1)	0
	Dimension (2)	0
as above alkaline pH	Hitachi 717 (2)	-49%
	Hitachi 717 (3)	-50%
	Hitachi 737	-19%
<i>Jendrassik</i> (DMSO) neutral pH	Open 30 (1)	-19%
	Open 30 (2)	-30%
Diphenyl diazonium	AU 510 (1)	-43%
	AU 510 (2)	+55%
	AU 5010 (1)	0
	Hitachi 717 (1)	0
	Hitachi 747	-78%
other diazo reagents	Chem 1 (1)	0
	Chem 1 (2)	0
	Dax 72	-36%
	Monarch (1)	0
	Monarch (2)	0
	Paramax (1)	+75%
	Paramax (2)	+20%
	Wako 30 R	+543%
Reflectometry	Ektachem (1)	+37%
	Ektachem (2)	+34%

Italic numbers = significant interference (according to the norms) at the highest level of interference

haemoglobin may be transformed to haematin or met-haemoglobin. At the same time, the bilirubin being measured is destroyed by haemoglobin peroxidase. On top

of this, the reaction kinetics of the blank, if used, may differ from the sample so that the interference will be positive without a blank and negative with a blank.

Bilirubin may also interfere as a reducing substance: as it is easily oxidised to biliverdin and bilipurpurin, it will react with all oxidants present. Many enzymatic tests used for measuring substrates use oxidases producing hydrogen peroxide. In the presence of peroxidase, different chromogens can be oxidised. Bilirubin may reduce the concentration of the final product proportionally to its own concentration by consuming the hydrogen peroxide. Here we may find an augmented effect due to the spectral changes in the medium caused by the oxidation of bilirubin (fig. 6). This explains why the results are systematically too low with the oxidase/peroxidase methods (tab. 14) for glucose, cholesterol, triacylglycerols and uric acid. This is also true for the enzymatic creatinine method on the Hitachi 717 (1) (tab. 6).

Lastly, *bilirubin oxidises spontaneously in an alkaline medium*, as in the *Jaffé* method for creatinine (tab. 12). This is probably the main reasons for the interferences found, rather than any spectral effects.

4.2 How do the instruments deal with this problem?

Basically, it is impossible to eliminate some kind of interferences – e. g. those caused by cell lysis. It is nevertheless possible to influence spectral and chemical interferences.

Tab. 14 Oxidase/peroxidase methods – The influence of bilirubin.

Instrument	Glucose		Cholesterol		Triacylglycerols		Uric acid	
	bilirubin	ditauro-bilirubin	bilirubin	ditauro-bilirubin	bilirubin	ditauro-bilirubin	bilirubin	ditauro-bilirubin
AU 5000 (2)	-43%	-20%	/	/	/	/	/	/
AU 5010 (1)	/	/	-42%	-15%	0	-25%	-11%	-19%
AU 510 (1)	/	/	-31%	-20%	0	-24%	0	-43%
AU 510 (2)	-21%	-14%	-33%	-19%	+19%	-45%	/	/
Chem 1 (1)	/	/	0	-13%	/	/	/	/
Chem 1 (2)	/	/	0	-19%	/	/	/	/
CX 7 (2)	/	/	-34%	/	-78%	/	/	/
Dax 72	-19%	-12%	?	-20%	0	0	-34%	-32%
Hitachi 717 (1)	-18%	0	/	/	/	/	-41%	-23%
Hitachi 717 (2)	-27%	0	-20%	-11%	0	0	-29%	-23%
Hitachi 737	0	0	0	0	0	0	-20%	-17%
Hitachi 747	-15%	/	-20%	/	-17%	/	-30%	/
Monarch (1)	-26%	-9%	-17%	0	0	0	0	-20%
Monarch (2)	-13%	0	-12%	-15%	0	0	0	-11%
Open 30 (1)	/	/	-17%	0	/	/	/	/
Open 30 (2)	/	/	-18%	0	-59%	-33%	/	/
Paramax (1)	/	/	-15%	-11%	/	/	-17%	0
Paramax (2)	/	/	-20%	-17%	/	/	-12%	0
Wako 30 R	-16%	/	-29%	/	-17%	/	0	/

Values in italics = significant interference (according to the norms) at the highest level of interference

4.2.1 Physical removal of impurities

This study confirms the previous one published by Glick in 1986 (5, 6) which showed that instruments using specimen pretreatment (strips with a layer for separation, dialysis) were best at eliminating both spectral and chemical interferences. In the same way, the SFBC method for iron chemically eliminates interference using trichloroacetic acid prior to carrying out the assay. The Kodak multilayer slides have a similar effect though, to a lesser extent, through the use of hydrophilic polymers on which the sample is deposited and which allow the passage of a set volume of aqueous liquid via capillaries. This blocks macromolecules and other substances which could interfere with measurement.

4.2.2 Operational conditions – Blank measurement

“Visible” interferences, particularly turbidity (4), may be minimised or even corrected by adjusting the reaction medium using e. g. a sample blank.

a) *blank measurement*: This involves measuring the sample absorbance, once suitably diluted, prior to adding the reagent. The absorbance measured (A1) is subtracted from the final absorbance (A2). There are several ways to realize this blank. The first method uses two different reagents (blank and reagent mixture) and two cuvettes. The second method is performed by sequentially adding the reagents which, however, is not always possible: some automated systems work only with a single reagent, or only have one position to dispense the reagents. Furthermore, these methods need two consecutive measurements on the same sample. Finally, a single reagent may be used with an early reading which is ranked as a blank, or with multiple readings to estimate the blank value. This, however, requires successive measurements on the same sample: if certain automated systems having several photometers have a limited number of measurements at fixed intervals, others systems only have one photometer. In that case, we will distinguish between the few automated systems which can only measure at the end point, and the many automated systems which, fortunately, can make several measurements. Here, the photometer may well be fixed, but the cuvettes will be transported in front of the photometer for readings at set intervals. If it is mobile, it will be able to read absorbances on all the cuvettes at the required times. These settings mean that many readings can be made for each sample and thus a blank value can be easily obtained.

This shows how important the concept of an instrument is, as well as the available algorithms, at different levels (treatment of the reaction medium, treatment of the readings, treatment of other data, e. g. the number of reagents per test, the number of readings per cuvette

Tab. 15 The interest of 2-cuvette blanking.

	Instrument	Turbidity	Sample blank
Iron	AU 510 (1)	0	yes, 2 cuvettes
	AU 510 (2)	<i>-100%?</i>	yes, sequential
Phosphorus	AU 510 (1)	0	yes, 2 cuvettes
	AU 510 (2)	<i>+127%</i>	no
	Instrument	Haemolysis	Sample blank
Bilirubin	Open 30 (1)	<i>-19%</i>	yes, 2 cuvettes
	Open 30 (2)	<i>-30%</i>	yes, 2 cuvettes

Values in *italics* = significant interference (according to the norms) at the highest level of interference

etc.). They both affect the quality of the results and the prevention or lack of prevention of interferences.

b) *blank preparation*: this also makes a difference (tab. 15). Better results are often obtained if 2 cuvettes with a blank are used rather than sequentially adding the reagents. When the blank develops slowly, automated systems are often not able to wait for its absorbance to stabilise before adding the reagent. Under these conditions, two cuvettes may be used, one containing all the reagents and the other containing all of them except one necessary for the reaction to take place. The blank is the one in which, after the reaction time, no colour is visible. The interference will be the same in both cuvettes.

In the study, several examples show that the use of 2 cuvettes produces excellent results. For example, on the 2AU 510, one used sequential blanking, the other 2 cuvettes. The results (fig. 8) show the advantage of 2 cuvettes. This is not the final solution for every test as

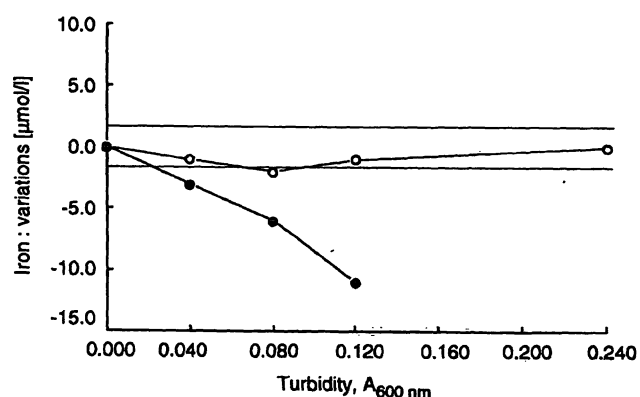


Fig. 8 The interest of 2-cuvette blanking – e. g. interference of turbidity on iron measurements on 2 AU 510 analysers using the same method and the same reagents; the first one AU 510 (1) works with a two cuvette blank, the second one AU 510 (2) with a sequential addition of reagents.
—○— AU 510 (1); —●— AU 510 (2)

shown in the results for bilirubin in the presence of haemolysis using 2 cuvettes: in this case, the chemical interference is difficult to remove (e. g. bilirubin on the Open 30 and the AU 510). The use of 2 cuvettes suffers one drawback reducing the throughput of tests by half, although generally improving the results overall.

4.2.3 Measuring conditions

Clinical chemistry analysers try, on the whole, to deal with *spectral interference* by acting on the readings made:

a) *choice of read wavelength*: the number of wavelengths available on automated systems is usually limited. They mostly use interference filters: some use light receptive arrays, but the number of diode receptors used is relative small (generally from 10 to 16). Thus, the wavelength used is not always the best. For example, the interference of bilirubin and haemolysis on uric acid measurement using the oxidase/peroxidase methods (tab. 16) varies with the wavelength.

b) *Multiwavelength analysis* appears attractive: since the interference may vary with time, corrections can be carried out at the end of the reaction by reading at different wavelengths and applying algorithms for correction. The easiest – and almost the only – method used in our experience in 1992, is *bichromatic* measurement. This involves using a secondary (side) wavelength to measure the interfering substance. The substance to be measured does not absorb at this wavelength. This measurement is then subtracted from that of the analyte required at its wavelength. This assumes that the absorbance of the interfering substance is the same at both wavelengths, which is rarely the case. Obviously, the bichromatic principle will not help unless the interfering substance does not absorb at the secondary wavelength. For

this reason, the corrections will either be partial or overestimated.

4.3 What can be obtained from the results?

By taking some relatively complex examples, we shall see that mastering “visible” interferences is very difficult if not impossible.

4.3.1 The uric acid case

Table 17 shows the results obtained: the instruments are listed by method, for the most part “uricase/catalase/dehydrogenase” and “uricase/peroxidase” with rarely “uricase 293 nm” and “uricase by reflectance photometry”.

Clearly, the “uricase/catalase/dehydrogenase” methods come out better than the “uricase peroxidase” methods which are often subject to interference (bilirubin and haemolysis in particular). Wherever possible on open systems, both manufacturers and end users should use the “uricase/catalase/dehydrogenase” methods. The influence of turbidity on the Chem 1 is surprising: the instrument uses sequential additions and there is probably a clarification of the reaction medium after the first reading.

For the “uricase/peroxidase” methods which very often use the *Trinder's* chromogen (4-aminophenazone and phenol or phenol derivatives), the choice of the secondary wavelength does not always appear to be the most appropriate. The same can be said for the other tests using these methods (glucose, cholesterol, triacylglycerols). Here we find virtually any wavelength (tab. 18). It seems that the best wavelength would be 560 nm, where haemoglobin and bilirubin absorbance is minimal, but the choice depends on the substrate used in the indicator reaction. It is also interesting to note that the sensitivity varies according to chromogen used – phenol bonded with halogens to varying degrees, *p*-chlorophenol, dichlorhydroxybenzene-sulphonic acid – which itself depends on the concentration of the substrate (glucose and cholesterol 5, triacylglycerols 2, uric acid 0.5). This leads to paradoxical results when considering that interferences – e. g. cholesterol and glucose – are more sensitive to bilirubin.

As for haemolysis, the uric acid test, using this method, suffers greater interference than other tests using the methods (cholesterol, glucose, triacylglycerols), which are diluted 1 : 100, whereas to improve sensitivity, uric acid is only diluted 1 : 50.

Finally, a sample blank only improves results where lipids are in excess.

Tab. 16 Uric acid, uricase *Trinder* methods: the influence of haemolysis and bilirubin at different wavelengths.

Instrument	Haemolysis	Total bilirubin	Wavelength (nm)
Hitachi 717 (1)	+15%	–41%	505
Hitachi 717 (2)	0	–29%	
Hitachi 747	+18%	–30%	
Dax 72	+31%	–34%	525
Paramax (1)	+16%	–17%	
Paramax (2)	+29%	–12%	
Monarch (1)	+58%	0	550
Monarch (2)	+27%	0	
Wako 30 R	+215%	0	
AU 510 (1)	+47%	0	570
AU 5010 (1)	+42%	–11%	
Hitachi 737	+36%	–20%	

Tab. 17 Uric acid measurement.

Method group	Instrument	Bilirubin	Ditauro-bilirubin	Haemolysis	Turbidity	Sample blank	Wavelength	
							main	side
Uricase/ catalase/ dehydrogenase	AU 5000 (2)	-22%	0	0	+12%	yes	340	380
	AU 510 (2)	0	0	0	+22%	yes ¹	340	380
	Chem 1 (1)	0	0	+13%	-61%?	yes	340	/
	Chem 1 (2)	0	0	0	-50%?	yes	340	/
	CX 7 (1)	0	0	-11%	-15%?	yes	340	380
	CX 7 (2)	0	/	0	-10%	yes	340	380
	Open 30 (1)	0	0	0	0	yes	340	/
	Open 30 (2)	0	0	-11%	0	yes	340	/
Uricase/ peroxidase	AU 5010 (1)	-11%	-19%	+42%	0	yes	570	750
	AU 510 (1)	0	-43%	+47%	0	no	570	800
	Dax 72	-34%	-32%	+31%	+50%	no	524	604
	Hitachi 717 (1)	-41%	-23%	+15%	0	yes	505	700
	Hitachi 717 (2)	-29%	-23%	0	0	no	505	/
	Hitachi 737	-20%	-17%	+36%	0	yes	570	660
	Hitachi 747	-30%	/	+18%	0	yes	505	/
	Monarch (1)	0	-20%	+58%	0	no	550	690
	Monarch (2)	0	-11%	+27%	-50%	no	550	690
	Paramax (1)	-17%	0	+16%	+19%	no	525	630
	Paramax (2)	-12%	0	+29%	+20%	no	525	630
	Wako 30 R	0	/	+215%	0	no	546	700
Uricase 293 nm	Dimension (1)	0	/	0	/	yes	293	700
	Dimension (2)	0	0	0	0	yes	293	700
Uricase/ reflectometry	Ektachem (1)	0	0	0	0	/	/	/
	Ektachem (2)	0	0	0	0	/	/	/

Italic numbers = significant interference (according to the norms) at the highest level of interference

Tab. 18 Oxidase/peroxidase methods – choice of main and side wavelengths.

Instrument	Glucose wavelengths (nm)	Cholesterol wavelengths (nm)	Triacylglycerols wavelengths (nm)	Uric acid wavelengths (nm)
AU 5000 (2)	540/600	/	/	/
AU 5010 (1)	/	540/600	520/600	570/750
AU 510 (1)	/	540/600	520/600	570/800
AU 510 (2)	520	540/600	520/600	/
Chem 1 (1)	/	500	/	/
Chem 1 (2)	/	500	/	/
CX 7 (2)	/	520/600	520/600	/
Dax 72	500/548	524/604	524/604	524/604
Hitachi 717 (1)	505/700	/	/	505/700
Hitachi 717 (2)	505	505	505	505
Hitachi 737	505/700	505/700	505/700	570/660
Hitachi 747	505	505	505	505
Monarch (1)	520	500/690	550/690	550/690
Monarch (2)	520/690	500/690	550/690	550/690
Open 30 (1)	/	510	/	/
Open 30 (2)	/	510	510	/
Paramax (1)	/	525/630	/	525/630
Paramax (2)	/	525/630	/	525/630
Wako 30 R	500/660	548/700	604/700	546/700

Italic numbers = significant interference (according to the norms) at the highest level of interference

4.3.2 The bilirubin case

In our study, this test was the most sensitive to interference (tab. 19); the assay suffered interference in 50% of cases (haemolysis: 68% – turbidity: 32%). Bilirubin is

apparently difficult to measure in a haemolysed specimen ... Thus blanking is needed (and is effective) to prevent lipaemic interference but does not influence haemolysis: using a blank produces a negative effect (bilirubin degradation?) and without one, the interference

Tab. 19 Bilirubin measurement.

Method group	Instrument	Haemolysis	Turbidity	Sample Blank	No. of reagents	Wavelengths (nm) main/side
<i>Jendrassik</i>	AU 5000 (2)	-60%	0	yes?	2	540/600
(benzoate caffeine)	CX 7 (1)	+47%	+31%	no	2*	560/600
neutral pH	CX 7 (2)	+33%	+19%	no	2*	560/600
	Dimension (1)	0	0	yes?	2	540/700
	Dimension (2)	0	0	yes?	2	540/700
as above alkaline pH	Hitachi 717 (2)	-49%	0	yes	2	546
	Hitachi 717 (3)	-50%	0	yes	2	546/660
	Hitachi 737	-19%	0	yes	2	546/660
<i>Jendrassik</i>	Open 30 (1)	-19%	0	yes, 2 cuvettes	1	550
(DMSO) neutral pH	Open 30 (2)	-30%	0	yes, 2 cuvettes	1	550
Diphenyl diazonium	AU 510 (1)	-43%	0	yes, 2 cuvettes	1	540/600
	AU 510 (2)	+55%	0	no	1	540/600
	AU 5010 (1)	0	0	yes	2	540/600
	Hitachi 717 (1)	0	-30%	yes	2	570/660
	Hitachi 747	-78%	0	yes	2	546/660
other diazo reagents	Chem 1 (1)	0	0	yes	2	550
	Chem 1 (2)	0	0	yes	2	550
	Dax 72	-36%	+25%	yes, 2 cuvettes	1	548/628
	Monarch (1)	0	+75%	no	2	550/620
	Monarch (2)	0	+78%	no	2	550/620
	Paramax (1)	+75%	+35%	no	1	550/630
	Paramax (2)	+20%	+46%	no	1	550/630
	Wako 30 R	+543%	+245%	?	2	604/700
Reflectometry	Ektachem (1)	+37%	0	/	/	/
	Ektachem (2)	+34%	0	/	/	/

DMSO = Dimethylsulphoxide

* the two reagents are added simultaneously

Values in italics = significant interference (according to the norms) at the highest level of interference

is positive; this is well illustrated by the results obtained on the AU 510 working with single reagents: system (1) uses a blank and has a negative interference; system (2) has none and has a positive interference. The choice of primary wavelength is also important: haemoglobin has 2 peaks at 540 and 575 nm, and 2 minimum absorbance at 509 and 559 nm (fig. 5). This interference is also seen on the Ektachem.

4.3.3 The case of iron measurement

Iron, in the present study, is very sensitive to interference (tab. 20): results are affected in 23.4% of cases overall (haemolysis: 64.6% - turbidity: 23.5%).

The interference caused by haemolysis is quite interesting since one micromole of haemoglobin (monomer) contains one micromole of iron. Thus, the final point of addition at 240 µmol/l of haemoglobin represents a large increase in iron in the specimen which would appear to make this test of no interest. However, for reasons we are unable to understand, there is no interference observed with certain reagents. In fact, this depends especially on the pH of the reaction medium and the de-

tergents used in the reagents: if the iron is not released but interference still exists, this is probably optical, and should not be seen if a sample blank is used; if the iron is released, it would be "normal" to see an interference of 1200%.

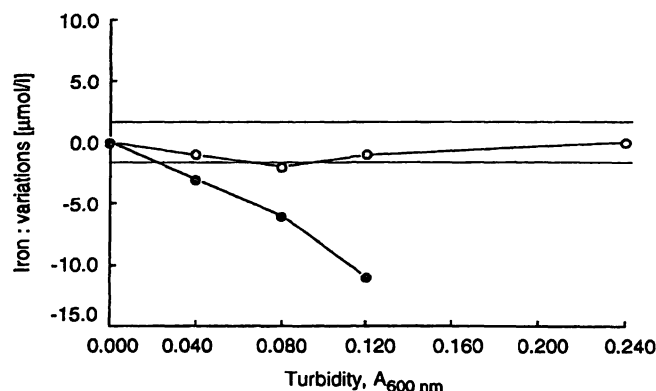


Fig. 8 The interest of 2-cuvette blanking - e. g. interference of turbidity on iron measurements on 2 AU 510 analysers using the same method and the same reagents; the first one AU 510 (1) works with a two cuvette blank, the second one AU 510 (2) with a sequential addition of reagents.
-○- AU 510 (1); -●- AU 510 (2)

Tab. 20 Iron measurement.

Method group	Instrument	Bilirubin	Ditauro-bilirubin	Haemolysis	Turbidity	Sample Blank	Wavelengths (nm) main/side
Ferrozine	AU 510 (1)	0	0	+81%	0	yes, 2 cuvettes	570/660
	AU 510 (2)	0	0	+64%	-100%?	yes, sequential	570/660
	CX 7 (2)	0	/	-50%	-14%	yes	560/700
	Hitachi 717 (3)	0	0	+94%	0	yes	570/700
	Hitachi 747	0	/	+13%	>-100%	yes	570
	Open 30 (2)	0	0	0	+17%	yes	550
	Paramax (1)	+10%	0	+62%	+9%	yes	550/630
	Paramax (2)	+17%	+14%	+49%	0	yes	550/630
	Wako 30 R	0	/	+295%	+13%	yes	572/700
Ferene	Chem 1 (1)	0	0	0	0	yes	600
	Chem 1 (2)	+12%	0	0	+15%	yes	600
	Dimension (1)	0	/	+187%	0	yes	600/700
	Dimension (2)	0	0	+150%	0	yes	600/700
	Hitachi 717 (2)	+14%	0	+140%	+74%	no	600
	Hitachi 737	0	0	+17%	+13%	yes	580/600
	Monarch (1)	0	0	+131%	+241%	no	

Italic numbers = significant interference (according to the norms) at the highest level of interference

Tab. 21 Phosphorus measurement using phosphomolybdate methods, reading at 340 nm: what using a sample blank adds.

Instrument	Bilirubin	Ditauro-bilirubin	Haemolysis	Turbidity	2-Reagent system	Side wavelength (nm)
AU 510 (2)	+14%	+41%	+58%	+127%	no	no
CX 7 (1)	0	+17%	+29%	+48%	no	600
CX 7 (2)	0	/	+40%	+74%	no	520
Hitachi 717 (1)	-57%	0	??	+53%	no	405
Monarch (1)	0	+20%	+24%	+61%	no	520
Open 30 (1)	+12%	+26%	+37%	+112%	no	no
Open 30 (2)	+17%	+29%	+37%	+78%	no	no
Paramax (1)	0	0	-17%	0	no	405
Paramax (2)	0	0	-20%	0	no	405
AU 510 (1)	0	+15%	+16%	0	no, 2 cuvettes	380
AU 5000 (2)	0	0	+21%	0	yes	380
AU 5010 (1)	0	0	-17%	0	yes	380
Chem 1 (1)	0	+12%	0	0	yes	no
Chem 1 (2)	0	0	0	0	yes	no
Dax 72	0	+28%	+17%	0	yes	548
Dimension (1)	+42%	/	-77%	0	yes (3)	383
Dimension (2)	+44%	0	-78%	0	yes (3)	383
Hitachi 717 (2)	0	0	0	0	yes	no
Hitachi 737	0	0	0	0	yes	660
Hitachi 747	0	/	0	0	yes	no
Waki 30 R	0	/	0	0	yes	660

Italic numbers = significant interference (according to the norms) at the highest level of interference

Looking at the turbidity effect, the results on the AU 510 show how the different types of blanking can affect results (fig. 8): system (1) uses a true blank with 2 cuvettes and the same read times; the interference due to turbidity is kept under control. System (2) blanks sequentially: a first reading is taken after a set time and reagent (2) is added, the reaction incubated and a second reading is made; the results are too low as the reaction medium clarifies over time.

4.3.4 The case of phosphorus

This test was found to be the second most sensitive in our study (tab. 21): results are affected in 34.1% of cases (haemolysis: 56.5% - total bilirubin: 13% - ditauro-bilirubin: 36.8% - turbidity: 30.4%). On most systems, the same method was used: measurement of the phosphomolybdate formed in an acid medium at 340 nm. However, this disguises many variations which are seen

in the operating conditions: results greatly vary when a single reagent is used; on the Hitachi 717, which uses 405 nm as secondary wavelength, all the interferences have an effect, whereas on the Paramax, which uses the same secondary wavelength, there is hardly no effect! The reagent composition (concentrations, surfactants and additives) certainly has something to do with this.

We were surprised to find that in 10 out of the 19 cases (52.6%) where bilirubin and ditauro-bilirubin were tested, ditauro-bilirubin interfered more than bilirubin (positive interference). Since the reaction takes place under extremely acidic conditions ($\text{pH} < 1$), not only does the accuracy suffer (hydrolysis of organic phosphoric esters), but, at this pH, the absorption spectra of the 2 bilirubin is altered as well (fig. 7).

On the whole, it seems better to work under the following conditions: use a 2-reagent system with a sample blank either using 2 cuvettes or with sequential addition; avoid using a secondary wavelength, or one as neutral as possible ($> 600 \text{ nm}$).

5 Conclusions

This study shows that results obtained from automated systems may not always be accurate in the presence of substances like haemoglobin or bilirubin or even when the specimen is cloudy in sufficiently high concentrations in the sample. We found that results were affected in 22.5% of cases under these conditions. The risk of interference is increased by the use of primary tubes often covered by several labels preventing visual inspections of the serum or plasma.

These results confirm those of Glick (5, 6) published in the United States in 1986 on the automated systems on the market at that time. It confirms the different publications and sessions at congresses, found in the literature, which have discussed interference problems. They indicate the same evolution that is seen in national and regional control schemes for quality control (External QC), whereby automation has improved precision considerably, but not accuracy as can be seen in the disper-

sion of the results obtained for the same test in all the laboratories.

The instrument manufacturers do employ corrective devices for interferences (blanking, bichromatic analysis). Most of these systems suffer imperfections making these corrections illusory. Available solutions however work. A few automated systems use physical methods (diffusion through a support material) to eliminate interfering substances, which is satisfactory for both chemical and spectral effects. On the other hand, progress in spectrophotometric measurement should yield good results even in the presence of coloured parasitic substances through the use of diode arrays (which can read spectra in a very short time) and computer software to treat the data obtained. These could easily be incorporated into the instruments (some already provide such equipment but do not use all the wavelengths).

Results obtained for clinical chemistry analysers can be improved in several ways: improve the preanalytic phase, use sample blanks, get optical measurements (bi or polychromatic procedures) under control. The precision on the automated systems is good and well mastered, much more so than the accuracy. This study illustrates the problems involved in accuracy and correction of interferences, as the results are very heterogeneous. There is only one way to treat chemical interferences. Eliminate the interfering substance: e. g. bilirubin with bilirubin oxidase, vitamin C with ascorbic oxidase.

Manufacturers try to satisfy their customers' needs, so users of automated systems have an important role to play. As soon as laboratory directors are convinced that they need accurate results, which indeed can and should improve, manufacturers will try to improve the automated systems. The choice of an automated system for the laboratory is always a big investment and will affect the quality of results. It should be as rigorous as possible in terms of quality.

Without the collaboration of the laboratory directors to carry out the tests along with the manufacturers' acceptance that their systems be tested, this study could not have been attempted. Our thanks to all and everyone concerned.

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